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Effect of Dosage on Immunogenicity of a Vi Conjugate Vaccine Injected Twice into 2- to 5-Year-Old Vietnamese Children†

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In a double-blind, randomized, and placebo-controlled previous trial, the efficacy of Vi-*r*EPA for typhoid fever in 2- to 5-year-olds was 89.0% for 46 months. Vi-*r*EPA contained 25 μg of Vi and induced a greater-than-eightfold rise in immunoglobulin G (IgG) anti-Vi in all of the vaccinees tested. In this investigation, we conducted a dosage-immunogenicity study of 5, 12.5, and 25 μg of Vi-*r*EPA in this age group. Two doses of Vi-*r*EPA were injected 6 weeks apart. Blood samples were taken before and at 10 weeks (4 weeks after the second injection) and 1 year later. All postimmunization geometric mean (GM) levels were higher than the preimmune levels ($P < 0.0001$). At 10 weeks, the GM IgG anti-Vi level elicited by 25 μg (102 EU/ml) was higher than those elicited by 12.5 μg (74.7 EU/ml) and 5 μg (43 EU/ml) ($P < 0.004$): all of the children had ≥3.52 EU/ml (estimated minimum protective level). One year later, the levels declined about sevenfold (13.3 and 11.3 versus 6.43 EU/ml, $P < 0.0001$) but remained significantly higher than the preimmune levels ($P < 0.0001$), and >96% of the children had a greater-than-eightfold rise. This study also confirmed the safety and consistent immunogenicity of the four lots of Vi-*r*EPA used in this and previous trials.

The Vi capsular polysaccharide of *Salmonella enterica* serovar Typhi is both an essential virulence factor and a protective antigen (1, 7, 11). To improve its immunogenicity in children <5 years of age, a conjugate composed of the Vi capsular polysaccharide bound to recombinant mutant *Pseudomonas aeruginosa* exoprotein A (Vi-*r*EPA) was synthesized (8, 13, 14). A double blind, randomized, and placebo-controlled trial with 2- to 5-year-old Vietnamese children showed Vi-*r*EPA to be safe and 89.0% effective in preventing typhoid fever for 46 months (9, 10). Among 76 children selected randomly from the phase 3 trial, Vi-*r*EPA induced a greater-than-eightfold rise in immunoglobulin G (IgG) anti-Vi in all vaccinees tested. The conjugate contained ~25 μg of Vi as Vi-*r*EPA. Because dosage-related immunogenicity has been shown for other conjugates such as the polysaccharides of *Haemophilus influenzae* type b and pneumococcus types, in this study the safety and immunogenicity of various dosages (5, 12.5, and 25 μg) of Vi as Vi-*r*EPA were evaluated in 2- to 5-year-old children (2, 3, 5, 6, 12).

MATERIALS AND METHODS

Study design. Two hundred forty-one children, 2 to 5 years old and evenly distributed by age and sex, were recruited from Thanh Ba District, Phu Tho Province, Vietnam. Half of the children were recruited from day care centers, and the other half were from the community of three communes. At the time of their recruitment and after informed consent was obtained from their parents or guardians, the children received identification numbers and were randomly assigned to three dosage groups (25, 12.5, and 5 μg of Vi as Vi-*r*EPA). Two injections of the same dosage were administered 6 weeks apart. Blood samples were collected before the first injection, 4 weeks after the second injection, and 1 year after the first injection.

Excluded were children with an illness requiring ongoing medical care, those who were immunocompromised, and those with a history of severe vaccine-associated reactions.

Before being injected, the children were examined by a physician and their axillary temperature was measured with a digital thermometer. Those with a temperature of ≤37.5°C received an injection in the deltoid muscle, and the vaccine code was recorded. The children were observed at 30 min by the vaccination teams and at 6, 24, and 48 h by a community health worker who recorded the axillary temperature and reactions at the injection site.

Vaccine. Vi-*r*EPA (lot 102970) and diluent (0.2 M NaCl, 0.01% thimerosal, and 10 mM sodium phosphate buffer at pH 7.0) were prepared by the National Institute of Child Health and Human Development (NICHD), National Institutes of Health (NIH), and bottled by the Pharmaceutical Development Section, Pharmacy Department, Clinical Center, NIH. Vi-*r*EPA, at 50 μg of Vi/ml, 2.9 ml/vial, met the requirements of the U.S. Code for Federal Regulations 610.

To maintain the 0.5-ml volume of conjugate for all injections, the 12.5- and 5-μg doses were prepared each day at the study site by dilution of the vials into color-coded vials and kept in cold boxes. Partially used vials at the end of each day were returned to the Typhoid Vaccine Study laboratory at Phu Tho Province and returned to the NICHD for analysis.

Serum IgG anti-Vi. Blood samples (2.0 ml) were collected by venipuncture, delivered into 2-ml centrifuge tubes, stored in a cold box until brought to the Typhoid Vaccine Study laboratory, and centrifuged, and the sera were collected. The sera were stored at −70°C and shipped in dry ice to the NICHD for blinded IgG anti-Vi titration by enzyme-linked immunosorbent assay (ELISA) (8). Results are expressed as the geometric mean (GM) and 25th and 75th percentiles (10). Comparisons of GMs were performed with the unpaired or paired *t* test.

This investigation was approved by the Institutional Review Board of the NICHD (OH98-CH-N002), NIH; the Center for Biologics Evaluation and Re-

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TABLE 1. Axillary temperatures after injection of 2- to 5-year-old Vietnamese children injected with 5, 12.5, or 25 μg of Vi as Vi-*r*EPA twice 6 weeks apart

Dose and temp (°C)	No. of children	First injection			No. of children	Second injection		
		6 h	24 h	48 h		6 h	24 h	48 h
5 μg	80				79			
≤37.5		80	80	80		78	78	79
37.6–38.5		0	0	0		1	0	0
38.6–39.5		0	0	0		0	1	0
12.5 μg	82				81			
≤37.5		80	80	82		80	80	81
37.6–38.5		2	1[a]	0		1	1	0
38.6–39.5		0	1	0		0	0	0
25 μg	79				78			
≤37.5		76	79	79		76	78	78
37.6–38.5		3	0	0		2	0	0
38.6–39.5		0	0	0		0	0	0

[a] This child had a temperature of 37.6°C at 6 h.

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RESULTS

Adverse reactions. There were no serious adverse reactions. Table 1 reports the temperatures of the vaccinees after the two injections. Elevated temperatures were infrequent, mild, and resolved within 24 h. After the first injection, a recipient of the 12.5-μg dose had a temperature of 39.0°C at 24 h. After the second injection, a recipient of the 5-μg dose had a temperature of 39.0°C.

None of the vaccinees had swelling or erythema at the injection site after the first injection. After the second injection, one recipient of the 25-μg dose had a 6-cm swelling for 24 h, 15 had erythema <1 cm in diameter at 6, 12, or 24 h. No local reactions were detected at 48 h.

After the second injection, three vaccinees complained of mild headache and one complained of nausea at 6 h: both of these symptoms subsided within 24 h.

Serum IgG anti-Vi. Table 2 lists the GM and 25th to 75th percentiles prior to the first injection, 4 weeks after the second injection, and 1 year after the first injection. One participant in each dosage group was lost to follow-up at 1 year. The antibody data are clear: there was a dose-related response, with the highest IgG anti-Vi levels elicited by the 25-μg dosage and the lowest elicited by the 5-μg dosage, although this difference was not statistically significant for the 25-μg dosage versus the 12.5-μg dosage at 1 year.

Four weeks after the second injection, all of the vaccinees had a ≥13-fold rise in IgG anti-Vi ($P < 0.0001$). The 25-μg dosage of Vi-*r*EPA elicited the highest level (102 EU/ml), the 12.5-μg dose elicited a level of 74.7 EU/ml, and the 5-μg dosage elicited a level of 43.0 EU/ml. The differences between all of these values are significant ($P < 0.004$). All recipients had ≥3.52 EU of IgG anti-Vi/ml, the estimated minimal protective level based on the efficacy trial (9).

The GM IgG anti-Vi levels declined at similar rates in all three groups during the first year: 6.7-fold in the 5-μg dose recipients (43.0 to 6.43 EU/ml), 6.6-fold in the 12.5-μg dose recipients (74.7 to 11.3 EU/ml), and 7.7-fold in the 25-μg dose recipients (102 to 13.3 EU/ml). At 1 year, 17 (23%) of the 75 5-μg dose recipients, 4 (5%) of the 79 12.5-μg dose recipients, and 4 (5%) of the 77 25-μg dose recipients had <3.52 EU of IgG anti-Vi/ml, the estimated minimal protective level (9).

TABLE 2. Serum IgG Vi antibodies elicited in 2- to 5-year-old Vietnamese children injected with Vi-*r*EPA[a]

Amt (μg) of Vi as Vi-*r*EPA	No. of children, GM no. of ELISA U/ml (25th–75th percentiles)[b]		
	Preimmune	10 wk	1 yr
5.0	76, 0.17 (0.10–0.22)	80, 43.0 (29.1–60.8)	75, 6.43 (3.84–10.4)
12.5	80, 0.14 (0.09–0.20)	80, 74.7 (49.9–102)	79, 11.3 (7.15–15.7)
25.0	78, 0.13 (0.08–0.20)	77, 102 (65.1–163)	77, 13.3 (7.87–23.3)

[a] Children were randomly assigned to three dosage groups (25, 12.5, and 5 μg of Vi as Vi-*r*EPA). Two injections of the same dosage were administered 6 weeks apart. Blood was collected before the first injection, 4 weeks after the second injection, and 1 year after the first injection. IgG anti-VI was assayed by ELISA (8).

[b] 43.0 versus 0.17, 74.7 versus 0.14, 102 versus 0.13, $P < 0.0001$; 102 versus 74.7, $P < 0.004$; 74.7 versus 43.0, $P < 0.0001$; 11.3 and 13.3 versus 6.43, $P < 0.001$; 13.3 versus 11.3, no significant difference.

DISCUSSION

As observed in three earlier trials with three separate lots, Vi-*r*EPA was safe and highly immunogenic (8–10, 14). Only 13 (2.7%) of 479 injections elicited a mild fever, which lasted no more than 24 h. Local reactions were similarly infrequent and mild.

It is our limited experience that larger polysaccharide doses of conjugates elicit antibodies of longer duration (4). We recommend the 25-μg dose of Vi as Vi-*r*EPA for 2- to 5-year-old children because this is the dose that gives the strongest antibody response and conferred protection for at least 4 years (9). Larger doses (>25 μg), although potentially more immunogenic, were not evaluated here.

In children <2 years of age, conjugates of *H. influenzae* type b and pneumococcus types had optimal immunogenicity at a dose of ~5 μg of polysaccharide (2, 4, 6). Because at 1 year in both the 12.5- and 25-μg dosage groups the GM IgG anti-Vi levels were not significantly different and 95% of the vaccinees had IgG anti-Vi levels considered to be protective, we plan to evaluate both doses of Vi-rEPA injected concurrently with diphtheria-pertussis-tetanus vaccine in infants for optimal immunogenicity, as well as the duration of IgG anti-Vi.

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